Scientific Abstract

An ideal vaccine for the prevention of cholera is not yet available. Previous work has resulted in the development of an attenuated live oral cholera vaccine, *V. cholerae* CVD 103-HgR. This vaccine confers strong protective immunity against experimental challenge with virulent *V. cholerae* O1 after a single dose. Although this vaccine is highly protective in U.S. volunteers and has been licensed in several developed countries for protection of travelers to cholera-endemic countries, a recent field trial of this vaccine in Indonesia failed to show efficacy in the native population of a cholera-endemic country. The development of attenuated cholera vaccines has been hampered by the fact that *V. cholerae* strains deleted of the *ctx* genes encoding cholera toxin can still produce varying amounts of diarrhea and non-diarrheal symptoms such as headache, fever, abdominal cramps, and malaise in many individuals. Such symptoms are not seen with CVD 103-HgR, probably because this strain colonizes the human intestine at greatly reduced levels compared to *ctx*-negative strains which are reactogenic and avid colonizers. The ability to construct a strain which colonizes the intestine better and therefore stimulates a more vigorous protective immune response is hindered by the uncertainty as to what bacterial factor is responsible for the reactogenicity of highly immunogenic, colonizing vaccine strains. The aim of this protocol is to use *in vivo* expression technology to identify *V. cholerae* O1 genes that are expressed *in vivo* in human volunteers. A library of *V. cholerae* O1 genes fused to a promoter-less *tnpR* gene in Ctx-negative El Tor Ogawa *V. cholerae* O1 strain CVD 110, consisting of a pool of 1 x 10^8 colony forming units in bicarbonate, will be given orally to up to 5 healthy adult inpatient volunteers. Volunteers will reside on a research isolation ward for 9 days after challenge to collect multiple specimens of stool and duodenal fluid for culture. *V. cholerae* isolates will be recovered from the stools and duodenal fluids, and the isolates will be screened for *in vivo* expression of the TnpR recombinase by loss of the *neo-sacB* genes. The identity of the cloned, *in vivo*-expressed genes will be determined by sequencing. This study is an opportunity to identify new *in vivo*-expressed virulence factors of *V. cholerae* and to ascertain new proteins that contribute to the protective immune response.